WEST Search History

DATE: Wednesday, October 02, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L57	L48 and L55	0	L57
L56	L51 and L55	0	L56
L55	withdrawl symptoms	3	L55
L54	drug addiction and withdrawl symptoms	0	L54
L53	sustance abuse and L51	0	L53
L52	drug addiction and L51	1	L52
L51	L48 and L50	159	L51
L50	L42 or L49	1232612	L50
L49	L39 or (L40 or L41)	3333	L49
L48	L43 or L44	441	L48
L47	L43 AND L44	39	L47
L46	L42 and L45	399	L46
L45	L39 and (L40 and L41)	480	L45
L44	(ginkgo biloba or ginkgo or maidenhair) near5 extract	374	L44
L43	ginkgolide or bilobalide or ((glycosylated or alkoxylated or acetylated) near5 ginkgolide)	106	L43
L42	treat$6	1231607	L42
L41	L40	1557	L41
L40	(substance or drug) near5 (dependence or abuse)	1557	L40
L39	(addict$6 or depend$4) near5 (alcohol or drug or amphetamines or tobacco)	2256	L39
DB=USPT,PGPB; PLUR=YES; OP=ADJ			
L38	L10 and drug addiction	2	L38
L37	L35 and drug addiction	3	L37
L36	L35 and substance abuse	0	L36
L35	L26 and L34	23	L35
L34	L23 and L24	23	L34
L33	L23 and L32	23	L33
L32	L22 and L31	23	L32
L31	L29 and L30	23	L31
L30	L28 and L25	23	L30
L29	L28 and L24	23	L29
L28	L26 and L27	23	L28

L27	L16 or L13	23	L27
L26	L16 or L14	23	L26
L25	L14 or L15	53	L25
L24	L13 or L15	53	L24
L23	L13 or L2	85	L23
L22	L14 or L2	85	L22
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L20	L13 and L2	0	L20
L19	L13 and L15	0	L19
L18	L13 and L16	0	L18
L17	L14 and L16	0	L17
L16	L2 and L15	20	L16
L15	L9 and L10	50	L15
L14	L8 and L13	3	L14
L13	L7 and L12	3	L13
L12	L3 and L5	1599	L12
L11	L9 and L10	50	L11
L10	(ginkgo biloba or ginkgo or maidenhair) near5 extract	219	L10
L9	ginkgolide or bilobalide or ((glycosylated or alkoxylated or acetylated) near5 ginkgolide)	98	L9
L8	(treat$6)	802665	L8
L7	(withdrawl symptoms)	5	L7
L6	(withdrawl symptoms) near5 (treat$6)	0	L6
L5	(substance or drug) near5 (dependence or abuse)	4027	L5
L4	substtance near5 (dependence or abuse)L3	0	L4
L3	(addict$6 or depend$4) near5 (alcohol or drug or amphetamines or tobacco)	13586	L3
DB=USPT; PLUR=YES; OP=ADJ			
L2	(((424/752)!.CCLS.))	82	L2
L1	((424/752)!.CCLS.)	82	L1

END OF SEARCH HISTORY

End of Result Set

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L38: Entry 2 of 2 File: USPT Aug 20, 2002

US-PAT-NO: 6436449
DOCUMENT-IDENTIFIER: US 6436449 B2

TITLE: Use of a composition

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gidlund; Bo	S-752 44 Uppsala			SE

APPL-NO: 09/ 796746 [PALM]
DATE FILED: March 2, 2001

PARENT-CASE:
This application claims priority from provisional application No. 60/186,356, filed Mar. 2, 2000.

INT-CL: [07] A61 K 35/78

US-CL-ISSUED: 424/752; 424/725, 424/773, 424/774, 424/775, 424/777, 424/94.1, 514/474
US-CL-CURRENT: 424/752; 424/725, 424/773, 424/774, 424/775, 424/777, 424/94.1, 514/474

FIELD-OF-SEARCH: 424/725, 424/752, 424/773, 424/774, 424/775, 424/777, 424/94.1, 514/474

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4543212	September 1985	Heinicke	
☐	5064858	November 1991	Sapse	
☐	5288491	February 1994	Moniz et al.	
☐	5840723	November 1998	Sands	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 98/35658	August 1998	WO	

OTHER PUBLICATIONS

Oscar Levand, Part 1. Some Chemical Constituents of Morinda Citrifolia L. (Noni), Thesis submitted to Graduate School of University of Hawaii, Jan. 1963.
O. A. Bushnell, et al., "The Antibacterial Properties of Some Plants Found in Hawaii", Pacific Science, vol. IV, pp. 167-183, Jul. 1950.
Alexandra Dittmar, "Morinda citrifolia L.-Use in Indigenous Samoan Medicine", Journal of Herbs, Spices & Medicinal Plants, vol. 1, No. 3, pp. 77-92, 1993.
Chafique Younos, et al., "Analgesic and Behavioural Effects of Morinda citrifolia", Planta Med., vol. 56, pp. 430-434, 1990.
Krupp M.A. and Chatton M.J., Current Medical Diagnosis and Treatment, 16.sup.th Annual Revision, p. 95, 1977.
Samuelsson G., Drugs of Natural Origin, A Textbook of Pharmacognosy, Swedish Pharmaceutical Press, pp. 47-51, and 294, 1999.
Anne Hirazumi, Antitumor Studies of a Traditional Hawaiian Medicianl Plant, Morinda Itrifolia (NONI), in Vitro and in Vivo, Dissertation submitted University of Hawaii for PhD in Biomedical Sciences (Pharmacology), Dec. 1997.

ART-UNIT: 1651

PRIMARY-EXAMINER: Prats; Francisco

ASSISTANT-EXAMINER: Coe; Susan D.

ABSTRACT:

Use of an extract derived from the fruits, leaves, the bark or the roots of Morinda citrifolia L. for the manufacture of a medicament for the treatment of a mammal suffering from tinnitus. The extract may be a liquid present in the medicament in an amount such as to give a daily dosage of 0.1-2 ml, or 0.2-1 ml, e.g. 0.4-0.7 ml, per kg body weight of the patient. The extract also may be a solid present in the medicament in an amount such as to give a daily dosage of 5-200 mg, or 10-100 mg, e.g. 20-70 mg, per kg body weight of the patient. Optionally, the medicament also may comprise lycopene, vitamine C, coenzyme Q10 and an extract from the leaves of Ginkgo biloba. The medicament may be given e.g. by oral, rectal, transdermal or inhalation administration.

18 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1 TECHNICAL FIELD

2 The present invention relates to the manufacture of a medicament for the treatment of a mammal suffering from tinnitus.

3 More specifically the present invention relates to the use of a composition comprising an extract from Morinda citrifolia L. (Rubiaceae) for the manufacture of such a medicament.

4 BACKGROUND ART

5 Morinda citrifolia L. (Rubiaceae), the Indian mulberry, also called noni, is an evergreen shrub tree which is native to Asia, Australia and some Pacific Islands. Its botanical description is given e.g. in Levand O. (Part I Some chemical constituents of Morinda citrifolia L (noni), thesis, University of Hawaii, 1963). The roots, bark, stem, leaves and fruits thereof have traditionally been used in medicine, in food and as a dye in different cultures, e.g. on Hawaii and in the French Polynesia. As an example, a plurality of indications of use is reported in the indigenous Samoan medicine, (Dittmar A."Morinda citrifolia L.--Use in Indigenous Samoan Medicine", J. of Herbs, Spices & Medicinal Plants, Vol. 1(3) pp 77-91 (1993)), covering a wide range of ailments, such as tooth ache (roots), septicemia (leaf), diarrhea of infants (bark) and eye complaints (fruit) . . . just to mention a few.

6 In view of the multiple traditional uses and alleged beneficial properties on human health of the plant, scientific studies have been undertaken to try to

identify the active principles in the different parts of the plant and to verify the medicinal effects obtained.

7 Thus, in view of a work by Bushnell et al. (Pacific Sci. 4, 167-83 (1950)) showing the antibacterial activity of the fruit of Morinda citrifolia (noni fruit), Levand (supra) tried to identify the chemical constituent of the fruit which would be responsible for this activity. A hypothesis was emitted that asperuloside, an aucubin-type glucoside found in an extract from the fruit, might have some antibacterial properties. Younos C. et al. ("Analgesic and Behavioural Effects of Morinda citrifolia", Planta Med. 56 pp.430-434 (1990)) investigated lyophilised aqueous extracts of roots of Morinda citrifolia for analgesic and behavioural effects in mice, finding a dose-related central analgesic activity as well as sedative properties at doses of 500-800 mg of dried plant material/kg of body weight.

8 Hirazumi A. et al. (Proc. West. Pharmacol. Soc. 37: 145-146 (1994)) studied the antitumour activity of juice extracted from noni fruits on intraperitoneally implanted Lewis lung carcinoma in syngenic mice, and found that the noni juice at a dose of 15 mg per mouse significantly increased the life span of the animals. The active substance was isolated by ethanol precipitation, but was not chemically identified.

9 In her thesis, Hirazumi further identified the antitumour active substance as a polysaccharide-rich substance. Appendix A of the thesis gives a list of the medicinal uses of the noni plant in traditional medicine in different regions of the world, and Appendix B gives a list of chemical constituents of the different parts of the noni tree. From Appendix B it can be seen that each part of the plant contains a varying number of different chemical constituents; in the fruits 66 different compounds are reported to have been found.

10 In an article intitulated "The pharmacologically active ingredient of noni" Heinicke, R. M. states that the active ingredient of the noni fruit in fact is not present in any substantial amount in the fruit itself, but is generated, on ingestion of the fruit or of an extract thereof, within the human body from a precursor present in the fruit. The active substance is said to be an alkaloid, which the author names xeronine, its precursor being named proxeronine. The author further emits some hypotheses on the biochemistry involved in the generation of the alkaloid from its precursor as well as on the mode of action of the alkaloid within the body, and finally recommends a daily intake of 100 ml of noni juice half an hour before breakfast. The physical conditions that might be favourably influenced are said to be e.g. high blood pressure, menstrual cramps, arthritis, gastric ulcers, sprains, injuries, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems, drug addiction, pain etc.

11 U.S. Pat. No. 4,543,212 (1985) to Heinicke relates to xeronine as a new alkaloid, and describes its characterization, assay, mode of action and utility within the medical, food and industrial fields. A process for obtaining xeronine from plant, bacteria and animal alkaloid producing lipophilic extracts is given. The activity of xeronine is stated to be due to its capacity to adhere to specific proteins as a modifier of rigidity of the same. The author notes that samples of xeronine acted as excellent anti-inflammatory agents when injected into mice, inhibited the in vitro aggregation of blood platelets by adenosine diphosphate, caused the debridement of burn eschars on mice, stimulated the partial breakdown of wheat grits and caused the aggregation of casein. Moreover, prediction is made that xeronine would be an effective antidote against alkaloid poisoning and addiction, and could be applied for the alleviation of symptoms of one type of senility and as a general stimulant or tonic. Finally, xeronine is also said to act as a coregulator for many hormone actions, a lack thereof thus being a possible cause contributing to e.g. diabetes.

12 U.S. Pat. No. 5,288,491 (1994) to Moniz relates to the noni plant as a medicinal product and teaches a method of processing the fruit into powder, mainly by picking, washing, cleaning and mashing the fruit, and then drying the pulp by thermal treatment in several steps and finally crushing and grinding the dried

wafers. The author refers to the paper by Heinicke and proposes that either pure xeronine or a system that releases xeronine be produced.

13 From the above, it appears that Morinda citrifolia L. has been used and recommended for use against an important number of diseases and ailments, and that a theory exists that an important active ingredient of at least the fruits of the plant is xeronine, which possibly may be present therein only in the form of its precursor.

14 Tinnitus is the perception of sound when no external sound is present; it is often referred to as "ringing in the ears." It can also take the form of hissing, roaring, whistling, chirping or clicking. The sensation may be objective (heard by the examiner) or subjective.

15 Objective tinnitus is uncommon and is caused by transmitted vascular vibrations in the blood vessels of the head and neck or by rythmic rapid contractions of the muscles of the soft palate or middle ear (Current medical diagnosis and treatment, 16th Annual revision, by Krupp M. A. and Chatton M. J. p.95 (1977)).

16 Subjective tinnitus is much less well understood. Although its etiology is at present not known, it is presumed to be due to irritation of nerve endings in the cochlea by degenerative vascular or vasomotor disease. It usually accompanies hearing loss or other disorders. The most frequent cause of tinnitus seems to be exposure to loud noise, either over an extended period of time or as one extreme incident.

17 The subjective form of tinnitus is very prevalent among adults. E.g. in a survey from Great Britain, about 10% of adults reported having prolonged, spontaneous tinnitus, with 1-3% reporting tinnitus severe enough to be disabling. Severe tinnitus is disabling due to the psychological effect of "hearing" sounds or noise continuously. Tinnitus prevents concentration, disrupts or prevents sleep, and in severe cases often leads to depression.

18 As stated in U.S. Pat. No. 5,840,723 (Sands), different modes of treatment are proposed to alleviate tinnitus, such as masking the noise by use of background music or "white noise" , relaxation training or medication. Medication has included intravenous administration of local anesthetics (lidocaine), trans-tympanic injections of local anesthetics, administration of zinc, steroids, anticonvulsants (carbamazepine), tranquilizers (alprazolam), barbiturates, antidepressants (trimipramine, nortryptyline), and calcium channel blockers (flunarizine), although in general with limited efficacy, or low acceptability due to the administration route in the case of the trans-tympanic injections. In the above cited U.S. Pat. No. 5,840,723 a treatment based on the use of quinoxaline derivates is disclosed.

19 Use of an extract from the leaves of Ginkgo biloba (Ginkgoaceae) as a herbal remedy for treatment of i.a. tinnitus has been proposed ("Drugs of Natural Origin, A Textbook of Pharmacognosy" by Samuelsson G., Swedish Pharmaceutical Press (1999) p.294). The leaves contain a class of diterpenes, called ginkgolides, as well as a sesquiterpene derivative, called bilobalide, and a great number of flavonoids. In the extract, the ginkgolides and the flavonoids are regarded as the pharmacologically active ingredients.

20 International application WO 98/35658 relates to the use of 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, also referred to as coenzyme Q10, as a pharmaceutical for the treatment of a multitude of indications, amongst others tinnitus. A capsule preparation containing from 30 mg to 120 mg of coenzyme Q10 is mentioned.

21 U.S. Pat. No. 5,064,858 relates to a composition for the treatment of individuals addicted to narcotics or individuals having age-related conditions such as tinnitus and Alzheimer's disease. The composition contains a protected complex of procaine and a complexing agent for procaine. Among a number of other additional compounds optionally present in the preparation, mention is made of zinc citrate and ascorbic acid, the former as a buffer and the latter as a

preferred complexing agent. Moreover, it is stated that a positive synergistic effect is believed to be obtained by the combination of procaine, ascorbic acid and zinc citrate as compared to the use of another selection of buffer and complexing agent.

22 SUMMARY OF THE INVENTION

23 The present invention is based on the discovery that an extract from Morinda citrifolia L. when administered to a person suffering from tinnitus will give a substantial relief of the ailment.

24 The present invention thus relates to the use of an extract from Morinda citrifolia L. for the manufacture of a medicament for the treatment of a mammal suffering from tinnitus, as well as to a method of treating a mammal suffering from tinnitus.

25 The extract may be derived from the fruits, the roots, the leaves and the bark of Morinda citrifolia L, and may be in a liquid or solid state.

26 The medicament may be in a form suitable for oral, rectal, inhalation or transdermal administration.

27 The medicament optionally may comprise one or more other active ingredients chosen from lycopene, an extract from the leaves of Ginkgo biloba, vitamin C and coenzyme Q10.

28 The combined use of two or more of the active ingredients as referred to above advantageously will give an enhanced antitinnitus effect.

29 DETAILED DESCRIPTION OF THE INVENTION

30 The extract from Morinda citrifolia L. may be derived from the roots, the bark, the leaves or the, fruits using extraction techniques well-known to the persons skilled in the art or described in the literature (e.g. in "Drugs of Natural Origin, A Textbook of Pharmacognosy" by Samuelsson G., Swedish Pharmaceutical Press (1999), cf. also references therein.).

31 The extract from the fruits may be either liquid, i.e. the juice as pressed from the fruits and treated in the way conventional to the art, or solid, i.e. dry, e.g. as a powder, e.g. as obtained by processing as described in U.S. Pat. No. 5,288,491 (1994) to Moniz.

32 As to the optional ingredients, lycopene, also referred to as .psi., .psi.-carotene or (all trans)-lycopene, is a carotenoid of C.sub.40 H.sub.56 occurring in ripe fruit, such as water melons and tomatoes. One kg of fresh ripe tomatoes may yield about 0.02-0.03 g lycopene. Carotenoids are nowadays thought to be highly efficient antioxidants. They are therefore being increasingly used e.g. in health products and in health food products to inactivate noxious free radicals generated in the human body. Also, there are studies showing that lycopene may be effective against different forms of cancers.

33 Due to concentrating effect of processing operations, such as cooking, where lycopene is not decomposed, the content of lycopene in processed tomato products is even higher than in the fresh tomatoes. Thus, the content of lycopene in 100 grams tomato ketchup amounts to around 10 mg, whereas the content of lycopene of the same amount of tomato juice is somewhat lower, around 9 mg, the content of lycopene of 100 grams of dried tomatoes being approximately the same, viz. around 9 mg.

34 Lycopene may also be synthesised by chemical or biosynthetic methods, or provided as a pure extract from a fruit or vegetable source, such as tomatoes. E.g. in U.S. Pat. No. 5,871,574 to Kawaragi et al. a process is provided for collecting tomato pigment.

35 In U.S. Pat. No. 5,962,756 to Koch et al. a process is provided for preparing

natural carotenoid concentrates from plant material.

36 On the other hand, in U.S. Pat. No. 5,965,183 to Hartal et al. a process is provided for the preparation of stable lycopene concentrates.

37 For the purpose of the present invention, lycopene may be included either in the form of a processed tomato product, such as tomato ketchup, tomato juice, dried tomato powder etc, or as a pure extract or synthetic product, or as a combination thereof. However, when lycopene is included in the form of a processed tomato product, such as tomato ketchup, this will result in a further advantageous effect of improving the general taste of the medicament.

38 When lycopene is present in the medicament of the invention, a suitable amount thereof will be one providing a daily dosage of 0.1 to 30 mg, or 0.2 to 15 mg, e.g. 0.5-5 mg, per kg body weight of the patient.

39 The extract from the leaves of Ginkgo biloba may be obtained by conventional extraction techniques, e.g. as described in Samuelsson G. (supra) pp 47 and references therein.

40 The further optional active ingredients, coenzyme Q10 and vitamin C, are commercially available or may be obtained by conventional synthesis or extraction methods known to the person skilled in the art.

41 The liquid extract from Morinda citrifolia will be present in the medicament in an amount such as to provide a daily dosage of 0.1-2 ml, or 0.2-1 ml, e.g. 0.4-0.7 ml, per kg body weight of the patient.

42 The dry extract from Morinda citrifolia will be present in the medicament in an amount such as to provide a daily dosage of 5-200 mg, or 10-100 mg, e.g. 20-70 mg, per kg body weight of the patient.

43 Optionally, vitamin C will be present in the medicament in an amount such as to provide a daily dosage of 0.05-20 mg, or 0.1-10 mg, e.g. 1-5 mg, per kg body weight of the patient.

44 Optionally, coenzyme Q10 will be present in the medicament in an amount such as to provide a daily dosage of 0.01-3 mg, or 0.1-1 mg, e.g. 0.2-0.5 mg, per kg body weight of the patient.

45 Optionally, the extract from the leaves of Ginkgo biloba will be present in the medicament in an amount such as to provide a daily dosage of 0.5-10 mg, or 1-7 mg, e.g. 2-5 mg, per kg body weight of the patient.

46 As an example, a daily dosage of a medicament comprising 10-100 ml of a liquid extract from Morinda citrifolia L., or 0.5-10 g of a dry extract from Morinda citrifolia L., and optionally 5-500 mg of vitamin C, and/or 1-150 mg of coenzyme Q10 and/or 10-500 mg of an extract from the leaves of Ginkgo biloba may be administered, as one single dose or subdivided into multiple doses.

47 The daily dosage will also depend on factors such as age, general state of health, severity of the tinnitus, other medication etc. It will also be appreciated that it in some cases may be sufficient to manage with less than the previously mentioned minimum amount, whereas the said upper limit in other cases may have to be exceeded.

48 Having regard to the particular mode of administration, the medicament of the invention, besides the active ingredient(s), may also incorporate any suitable additive, adjuvant and excipient as conventionally used within the pharmaceutical field, provided these do not unduly interfere with the active ingredients.

49 The medicament in a form suitable for oral administration may be e.g. a liquid solution, emulsion or suspension, granules, a pill, a capsule, a tablet etc, to be administered in a single daily dose or as several daily doses. Additionally,

the medicament may contain a solvent, a filler, a flavouring agent, a disintegrant, a preservative, a colouring agent etc as known to the persons skilled in the art.

50 Furthermore, the medicament for oral administration may be a sustained release preparation such as a depot tablet or capsule.

51 The medicament in a form suitable for rectal administration may be a suppository incorporating any suitable formulating aid, suppository base, a glidant etc, as known to the persons skilled in the art.

52 For transdermal administration the medicament of the invention may be incorporated into a plaster, using methods and materials well known to the persons skilled in the art.

53 For the inhalation administration, the medicament of the invention may be formulated into an aerosol preparation.

54 It will be understood that the administration forms mentioned above are only examples, and that other modes of administration may also be contemplated by the person skilled in the art without departing from the scope of the invention.

55 The medicament of the invention is prepared by processing of a composition of active ingredient(s) and any suitable excipents, in a conventional manner corresponding to the selected administration form, as known to the persons skilled in the art or as described e.g. in the European Patent Application 0 208 235.

DETAILED DESCRIPTION:

1 EXAMPLE

2 A male patient, aged 87, who for several years had been suffering from a severe tinnitus, was given a liquid extract from the fruit of Morinda citrifolia at a daily dosage of 30 ml, corresponding to a daily dosage per kg body weight of 0.4 ml.

3 At the start of the treatment period, the patient experienced a constant whistling and roaring noise in the ears.

4 After 14 days of treatment, the patient experienced that a reduction of the noise in the ears was taking place.

5 After 1 month of treatment, the patient estimated that the noise was very disturbing during 75% of the time of the day, and less disturbing during the remaining 25% of the time of the day. Moreover, the patient estimated that the maximum intensity of the noise had been reduced to 65-75% of its initial maximum value.

6 After 2 months, the period of time during which the patient experienced less disturbance from the noise had increased to 50% of the day.

7 After 3 months, the patient now estimated that the noise was disturbing during 25% of the time of the day, and less disturbing during the remaining 75% of the time of the day, and that the maximum intensity of the noise had been substantially reduced.

8 After 5 months of treatment, the noise was experienced as disturbing only occasionally, i.e. once or twice a month.

9 After 8 month of treatment, the patient experienced only intermittently a very faint sound in the ears.

CLAIMS:

What is claimed is:

1. A method of treating tinnitus in a mammal comprising administering to a mammal in need of such treatment a therapeutically effective amount of an extract derived from the fruits, the leaves, the bark or the roots of Morinda citrifolia L.

2. A method according to claim 1, wherein the extract is administered in the form of a medicament composition which further comprises at least one additional active ingredient selected from the group consisting of lycopene, vitamin C, coenzyme Q10 and an extract from the leaves of Ginkgo biloba.

3. The method according to claim 1, wherein said extract is in liquid form.

4. The method according to claim 1 wherein said therapeutically effective amount is at a daily dosage of 0.1-2 ml per Kg body weight of said mammal, and said mammal is a human patient.

5. The method of claim 4, wherein said daily dosage is 0.4-0.7 ml per Kg body weight of said patient.

6. The method of claim 1 wherein said extract is in solid form.

7. The method according to claim 6 wherein said therapeutically effective amount is at a daily dosage of 5-200 mg per Kg body weight of said mammal, and said mammal is a human patient.

8. The method of claim 7, wherein said daily dosage is 20-70 mg per Kg body weight of said patient.

9. The method of claim 1 wherein said extract is in a form suitable for oral, rectal, inhalation, or transdermal administration, and is administered orally, rectally, by inhalation or transdermally.

10. The method of claim 2 wherein lycopene is present in said medicament composition and is administered in an amount to give a daily dosage of lycopene of 0.1-30 mg per Kg body weight of the mammal, and the mammal is a human patient.

11. The method of claim 10 wherein said amount of lycopene is 0.5-5 mg per Kg body weight of said patient.

12. The method of claim 2 wherein vitamin C is present in said medicament composition and is administered in an amount to give a daily dosage of vitamin C of 0.05-10 mg per Kg body weight of the mammal, and the mammal is a human patient.

13. The method of claim 12 wherein said amount of vitamin C is 1-3 mg per Kg body weight of said patient.

14. The method of claim 2 wherein coenzyme Q10 is present in said medicament composition and is administered in an amount to give a daily dosage of coenzyme Q10 of 0.01-3 mg per Kg body weight of the mammal, and the mammal is a human patient.

15. The method of claim 14 wherein said amount of coenzyme Q10 is 0.2-0.5 mg per Kg body weight of said patient.

16. The method of claim 2 wherein extract from the leaves of Ginkgo biloba is present in said medicament composition and is administered in an amount to give a daily dosage of extract from the leaves of Ginkgo biloba of 0.2-10 mg per Kg body weight of the mammal, and the mammal is a human patient.

17. The method of claim 16 wherein said amount of extract from the leaves of Ginkgo biloba is 2-5 mg per Kg body weight of said patient.

18. The method of claim 2 wherein said medicament composition contains two of said additional active ingredients, three of said additional active ingredients, or all four of said additional active ingredients.

L35: Entry 19 of 23 File: USPT Mar 21, 1995

US-PAT-NO: 5399348
DOCUMENT-IDENTIFIER: US 5399348 A

TITLE: Extract from Ginkgo biloba leaves, its method of preparation and pharmaceuticals containing the extract

DATE-ISSUED: March 21, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schwabe; Klaus-Peter	Karlsruhe			DE

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Dr. Willmar Schwabe GmbH & Co.	Karlsruhe			DE	03

APPL-NO: 07/ 905167 [PALM]
DATE FILED: June 24, 1992

PARENT-CASE:
This application is a continuation of application Ser. No. 07/625,729, filed on Dec. 4, 1990, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	39 40 091.3	December 4, 1989

INT-CL: [06] A61 K 35/78, A61 K 31/70

US-CL-ISSUED: 424/195.1; 514/27
US-CL-CURRENT: 424/752; 514/27

FIELD-OF-SEARCH: 424/195.1, 514/27

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
	4708949	November 1987	Liu	514/26
	4753929	June 1988	Matsumoto	514/27
	4886904	December 1989	Tanaka	560/249
	4981688	January 1991	Ayroles	424/195.1

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FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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ART-UNIT: 185

PRIMARY-EXAMINER: Wityshyn; Michael G.

ASSISTANT-EXAMINER: Gitomer; Ralph

ABSTRACT:

The invention relates to an improved extract from Ginkgo biloba leaves, a method of preparation of the same and pharmaceuticals containing the extract.

16 Claims, 0 Drawing figures
Exemplary Claim Number: 7

BRIEF SUMMARY:

1 The invention relates to an improved extract from Ginkgo biloba leaves, a method of preparation of the extract and the pharmaceuticals containing the extract.

2 Extracts from the leaves of Ginkgo biloba have been used for a long time for the therapy of peripheral and cerebral arterial circulatory disturbances. Methods of preparation of Ginkgo biloba extracts with a greatly enriched content of flavone glycosides as the active components are known; see DE-B 17 67 098 and DE-B 21 17 429. These extracts are also referred to as Ginkgo biloba monoextracts.

3 EP-A 0 324 197 describes a method of preparation of an extract from Ginkgo biloba leaves in which an aqueous solution of a lower alcohol or ketone, obtained after extraction of the leaves, is concentrated in the presence of kieselguhr. The resultant aqueous suspension is filtered through kieselguhr, the fitrate is extracted with butanone and the extract is freed from the solvent.

4 EP-A 330 567 relates to a method of preparation of an extract from Ginkgo biloba leaves in which the crushed leaves are extracted with an aqueous ketone compound. This extract is concentrated until biflavones and hydrophobic compounds precipitate. After filtration the aqueous concentrate is rendered alkaline, whereby the proanthocyanidins precipitate.

5 After separation of the precipitate and acidification of the filtrate, a liquid-liquid-extraction is carried out with a C.sub.4-6 -ketone compound in the presence of ammonium sulfate. The extract is obtained after stripping of the ketone compound.

6 DE-A 35 14 054 has disclosed that the ginkgolides, known components of the

Ginkgo biloba leaves which are terpenoid substances with lactone structure (see K. Nakanishi, Pure and Applied Chemistry, Vol. 14 (1967), 89-113, and M. Maruyama et al., Tetrahedron Letters (1967), 299-302 and 303-319, and K. Okabe et al., J. Chem. Soc. (1967), 2201-2206), can be used to treat illnesses and similar conditions caused by PAF ("Platelet Activating Factor").

7 The use of bilobalide, a further substance contained in the Ginkgo biloba leaves, is known from DE-A 33 38 995 and the corresponding U.S. Pat. No. 4,571,407 for the treatment of demyelinating neuropathies, encephalopathies and cerebral edemas. Bilobalide is a sesquiterpene lactone structurally related to ginkgolides (see K. Nakanishi et al., R. T. Major et al., and K. Weinges et al., J. Am. Chem. Soc., Vol. 93 (1971), 3544-3546).

8 Besides the compounds mentioned above, Ginkgo biloba leaves also contain so-called ginkgolic acids (anacardic acids). These compounds are 6-alkylsalicylic acids with n-C.sub.13 - to n-C.sub.19 -alkyl groups with 0 to 3 double bonds; see J. L. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961 and Analytic. Chem., Vol. 40 (1968), 739-743.

9 "Ginkgol", a phenol substituted with the corresponding alkyl group, can be obtained either biogenetically by decarboxylation of the ginkgolic acids or during the technical processing of the Ginkgo biloba leaves; see Kawamura, Japan, J. Chem., Vol. 3 (1928), 91-93.

10 The ginkgolic acids and ginkgols in Ginkgo biloba are accompanied by corresponding derivatives with a further phenolic hydroxyl group in 4-position, the 6-alkylresorcylic acids or 5-alkylresorcins; see J. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961. These resorcin derivatives are responsible for the toxic effects and especially for the strong allergies and contact dermatitis caused by toxicodendron plants; see G. A. Hill et al., J. Am. Chem. Soc., Vol. 56 (1934), 2736-2738.

11 Cases of strong allergic reactions after contact with Ginkgo fruits are known; see W. F. Sowers et al., Arch. Dermatol., Vol. 91 (1965), 452-456, and T. Nakamura, Contact Dermatitis, Vol. 12 (1985), 281-282. Serious mucosal disturbances after eating Ginkgo fruits have been described; see L. E. Becker and G. B. Skipworth, J. Am. Med. Assoc., Vol. 231 (1975), 1162-1163. Allergic skin reactions also occur occasionally on collecting or handling Ginkgo leaves.

12 The significance of allergies caused by alkylphenol compounds from anacardiaceae and ginkgoaceae is evident from the development of substances and methods of desensitisation described in patent literature (see U.S. Pat. No. 4,428,965) against the allergies caused by alkylphenol compounds.

13 Commercial extracts from Ginkgo biloba leaves contain between 50 and 10,000 ppm ginkgolic acids.

14 The extracts from Ginkgo biloba leaves prepared by the known methods in DE-B 17 67 098 and DE-B 21 17 429 are substantially free of alkylphenol compounds because the lipophilic components of the extract are removed by a liquid-liquid-extraction of the aqueous acetone extract with a substantially water-immiscible lipophilic solvent, e.g. with a chlorinated aliphatic lower hydrocarbon such as carbon tetrachloride. However, in this step, the therapeutically valuable ginkgolides and the bilobalide are also considerably reduced so that their content in the final product in Example 1 of DE-B 21 17 429 is a maximum of 0.5% in the case of ginkgolides A, B, C and J in total and approximately 0.3% in the case of bilobalide. The quantity of flavone glycosides, however, is greatly increased during this step, namely from 3 to 4% in the crude extract to approximately 24% in the final product.

15 The object of the present invention therefore is to provide an extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds, has a high content of flavone glycosides and which contains substantially all of the ginkgolides and bilobalide present in the leaves used.

16 A further object of the invention is to provide a method of preparation of the extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds and which has a high content of flavone glycosides, ginkgolides and bilobalide. The method of the present invention should, in contrast to the known methods in DE-B 17 67 098 and DE-B 21 17 429, succeed in removing the alkylphenol compounds without the use of chlorinated aliphatic hydrocarbons. The use of chlorinated hydrocarbons in technical processes is very problematic because of the occupational medical risks, the potential danger of these compounds to the environment and the possibility of undesirable residues in pharmaceuticals.

17 Finally, it is the object of the invention to provide pharmaceuticals which contain this Ginkgo biloba extract with a high content of flavone glycosides, ginkgolides and bilobalide and where there is substantially no danger of allergic reactions, precisely because of the removal of the alkylphenol compounds.

18 The invention therefore relates to an extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds, which has a high flavone glycoside content and which contains most of the ginkgolides and the bilobalide originally present in the leaves. Preferably the extract in the present invention should contain

19 20 to 30 weight percent, in particular 22 to 26 weight percent, flavone glycosides,

20 2.5 to 4.5 weight percent of ginkgolides A, B, C and J (in total),

21 2.0 to 4.0 weight percent bilobalide,

22 less than 10 ppm, in particular less than 1 ppm, alkylphenol compounds and

23 less than 10 weight percent proanthocyanidins.

24 In addition, the invention relates to a method of preparation of this Ginkgo biloba extract from Ginkgo biloba leaves.

25 More specifically, a method is described for the preparation of an extract from the leaves of Ginkgo biloba which is substantially free of alkylphenol compounds and has a high flavone glycoside content and a content of ginkgolides and bilobalide which corresponds to most of these components originally present in the leaves. The method comprises an extraction of the leaves with aqueous acetone, and aqueous alkanol of 1 to 3 C-atoms or anhydrous methanol. The lipophilic components are removed by at least one treatment with ammonium sulfate and a subsequent extraction with methylethylketone or a mixture of methylethylketone and acetone, as well as a treatment with a lead compound or an insoluble polyamide. The method is characterized in that most of the organic solvent is separated from the extract from the leaves containing the aqueous organic solvent, and the remaining aqueous solution is diluted to a solids content of 5 to 25 weight percent, preferably approximately 15 to 20 weight percent, and left to cool and stand until a precipitate forms from the lipophilic components which do not dissolve well in water. This precipitate is then separated, and the aqueous alcohol solution obtained following the treatment with the lead compound is extracted with an aliphatic or cycloaliphatic solvent with a boiling point of approximately 60.degree.-100.degree. C. in order to further separate the alkylphenol compounds.

26 Furthermore, a method is described for the preparation of an extract from Ginkgo biloba leaves, containing: 20 to 30 weight percent, preferably 22 to 26 weight percent, flavone glycosides; 2.5 to 4.5 weight percent ginkgolides A, B, C and J (in total); 2.0 to 4.0 weight percent bilobalide; less than 10 ppm, preferably less than 1 ppm, alkylphenol compounds; and less than 10 weight percent proanthyocyanidins. This method is characterized in that the fresh or dried green leaves of Ginkgo biloba are extracted at a temperature of approximately

40.degree. to 100.degree. C. with aqueous acetone, an aqueous alkanol of 1 to 3 C-atoms, or anhydrous methanol. Most of the organic solvent is then separated from the extract to a maximum content of 10 weight percent, preferably a maximum of 5 weight percent, whereby water can be added in the last steps of distillation. The remaining concentrated aqueous solution is diluted with water to a solids content of 5 to 25 weight percent, preferably 15 to 20 weight percent, and left to cool, while being stirred, to a temperature below 25.degree. C., preferably approximately 10.degree. to 12.degree. C. The solution is left to stand until a precipitate forms and the resultant precipitate, consisting of the lipophilic components which do not dissolve well in water, is removed. The extract obtained is concentrated to a solids content of 50 to 70%, and the concentrate obtained is diluted with water and ethanol so that a solution is obtained which contains 50 weight percent of water and 50 weight percent of ethanol with a solids content of 10 weight percent. An aqueous solution of a lead salt such as lead acetate, lead hydroxide acetate or lead nitrate, or an aqueous suspension of lead hydroxide, preferably a solution of lead hydroxide acetate, is added to the thus obtained solution until a change in color from brown to umber takes place, and the precipitate formed is removed. The remaining aqueous-alcohol solution is extracted with an aliphatic or cycloaliphatic solvent with a boiling point of approximately 60.degree. to 100.degree. in order to further remove the alkylphenol compounds. The remaining aqueous-alcohol solution is concentrated under reduced pressure to a minimum ethanol content of approximately 5% and ammonium sulfate is added up to a content of 20 weight percent. The solution obtained is extracted with a mixture of methylethylketone and ethanol in a ratio of 9:1 to 4:6, preferably 4:6. The resultant organic phase is concentrated to a solids content of 50 to 70 weight percent. Finally, the resultant concentrate is dried under reduced pressure at a maximum temperature of 60.degree. to 80.degree. C. to a dry extract with a water content of less than 5%. Or, instead of a lead salt, a polyamide such as polyamide-6, polyamide-6,6 or cross-linked polyvinyl pyrrolidone (Polyvidon) can also be used. In contrast to the method of separating the lipophilic components described in DE-B 17 67 098, the aqueous alcohol or aqueous acetone crude extract is not directly subjected to liquid-liquid-extraction with a chlorinated aliphatic hydrocarbon, but rather most of the lipophilic components, which precipitate on distillation of the organic solvent components and dilution with water to a maximum content of 10 weight percent, preferably 5 weight percent, are separated by filtration. The alkylphenol compounds, the chlorophyll, the fatty acid derivatives and the biflavones precipitate due to their lower solubility in water and can be separated by filtration. Under these conditions, the desired components of the Ginkgo biloba extract remain dissolved. The alkylphenol compounds are reduced further to a content of less than 10 ppm in a subsequent degreasing step.

27 The extract obtained by extraction of the aqueous solution with methylethylketone/acetone, according to Example 5 in DE-B 17 67 098, is freed from the solvent by distillation. The residue is dissolved in 20 to 60 percent of aqueous ethanol until it has a solids content of 5 to 20%, preferably approximately 10%, and to this solution is added an aqueous solution of a lead salt, as in Example 1 and 2 in DE-B 21 17 429. After separation of the lead precipitates, the aqueous ethanol solutions obtained can be subjected either directly or after dilution with water to a ethanol content of preferably 30%, to a multistage liquid-liquid-extraction with an aliphatic or cycloaliphatic hydrocarbon (boiling point of approximately 60.degree. to 100.degree. C.). The filtrate obtained according to Example 3 (DE-B 21 17 429) can likewise be used either directly or after adjusting the ethanol content to approx. 30%.

28 In pharmacological experimental models, the extract prepared according to the present invention has radical scavenging properties and properties which stimulate the circulation of blood, prevent ischemic disorders and inhibit platelet aggregations.

29 In addition, the invention relates to pharmaceuticals which are characterized by a content of Ginkgo biloba extract.

30 The Ginkgo biloba extract of the invention can be processed in the usual way for

the preparation of pharmaceuticals e.g. to solutions, coated tablets, tablets or injection preparations. The pharmaceuticals in the invention are used for the treatment of peripheral and cerebral arterial circulatory disturbances. The examples illustrate the invention. Parts and percentage data refer to weight unless otherwise stated.

DETAILED DESCRIPTION:

1 EXAMPLE 1

2 100 kg of dry Ginkgo biloba leaves are crushed in a mill to a particle size of less than 4 mm. After adding 750 kg of 60 weight percent aqueous acetone the mixture is stirred intensively for 30 minutes at a temperature of 57.degree. to 59.degree. C. The solid residue is separated by filtration or centrifugation and subjected to a second extraction under the same conditions. The extracts from the first and second extraction steps are combined. The ginkgolic acid content (based on the dry extract) equals approximately 13,000 ppm. The extract is concentrated under reduced pressure to a solids content of 30 to 40% and a maximum of approximately 5 weight percent acetone. By adding water, the concentrate is diluted to double volume and, while being stirred, left to cool to approximately 12.degree. C. A precipitate forms which contains most of the ginkgolic acids, that is, the alkylphenol compounds, present in the leaves. After one hour at this temperature, the resultant precipitate is separated by centrifugation and discarded.

3 The ginkgolic acid content in the resultant aqueous supernatant (based on the dry extract) equals approximately 320 ppm.

4 30 parts of ammonium sulfate are added to 100 parts of the aqueous solution. The mixture is stirred. After the ammonium sulfate has dissolved, a liquid-liquid-extraction is carried out twice with a mixture of methylethylketone and acetone in a ratio of 6:4 to 1:1, whereby the organic solvent added is the equivalent of half the volume of the aqueous solution and, after intensive stirring and pumping, the organic upper phase formed on completion of the mixing process is removed.

5 The methylethylketone acetone solution is then concentrated under reduced pressure to a solids content of 50 to 70%. This concentrate is diluted with water and 95 weight percent ethanol so that a solution with 10 weight percent dry extract in 50 weight percent aqueous ethanol is obtained. While stirring intensively, an aqueous solution of lead hydroxide acetate is added in small quantities to this solution until there is a change in colour from brown to umber (brown with a green cast). The lead-tannin precipitate which forms is separated by centrifugation.

6 The supernatant from the lead-tannin precipitation is subjected to a liquid-liquid-extraction with n-hexane in order to further remove the alkylphenol compounds. In this step, the alcohol-aqueous filtrate is stirred at least three times at room temperature, each time with 1/3 of its volume of n-hexane.

7 The aqueous-alcohol extract solution is then concentrated under reduced pressure to an ethanol content of less than approximately 5%. 20 parts of ammonium sulfate are dissolved in 100 parts of this solution and then a liquid-liquid-extraction is carried out with a mixture of methylethylketone and ethanol in a volumetric ratio of 6:4, whereby extraction with the organic solvent mixture is carried out twice, each time with the equivalent of half the volume of the aqueous solution. The organic phase is separated and stirred with 20% of its weight of ammonium sulfate. A possible phase of water and the undissolved ammonium sulfate are removed.

8 The clear extract solution is concentrated to a solids content of 50 to 70 weight percent. This concentrate is dried under reduced pressure at a maximum

product temperature of approximately 60.degree. to 80.degree. C. to a dry extract with a water content of less than 5%.

9 From 100 kg of Ginkgo leaves, 2.5 kg of Ginkgo biloba extract with a content of approximately 24 weight percent flavone glycosides, approximately 3.6 weight percent ginkgolides, approximately 2.9 weight percent bilobalide, approximately 6.5 weight percent proanthocyanidins and less than 1 ppm alkylphenol compounds are obtained.

10 EXAMPLE 2

11 Solution for oral administration:

12 100 ml solution contains:

Ginkgo biloba extract	4.0 g
ethanol	50.0 g
demineralised water to	100.0 ml

13 EXAMPLE 3

14 Coated tablets:

15 1 tablet contains:

Ginkgo biloba extract	40.00 mg
microcrystalline cellulose	100.00 mg
lactose	80.00 mg
colloidal silicic acid	25.00 mg
talcum (in core)	4.50 mg
magnesium stearate	0.50 mg
hydroxypropyl methylcellulose	12.00 mg
ferric oxide pigment	0.10 mg
talcum (in coat)	0.50 mg
weight of a coated tablet	approx. 262.60 mg

CLAIMS:

I claim:

1. An extract comprising 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent of ginkogolides A, B, C and J, 2.0 to 4.0 weight percent bilobalide, less than 10 ppm alkylphenol compounds and less than 10 weight percent proanthocyanidins.

2. The extract of claim 1 containing about 22 to 26% by weight flavone glycosides.

3. The extract of claim 1 containing less than 1 ppm alkylphenol compounds.

4. A pharmaceutical composition useful for the treatment of peripheral and

cerebral arterial circulatory disorders comprising a Ginkgo biloba extract of claim 1 in a pharmaceutical carrier wherein the extract concentration is sufficient to alleviate the circulatory disorders.

5. A method of preparing an extract from the leaves of Ginkgo biloba which is substantially free of alkylphenol compounds and having a high content of flavone glycosides and comprising substantially all of the ginkgolides and bilobalide originally present in the leaves, the method comprising the steps of

a) extracting the leaves with an organic solvent selected from the group consisting of aqueous acetone, an aqueous alkanol having one to three carbon atoms and anhydrous methanol;

b) separating most of the organic solvent from the extract of step (a) to form an aqueous solution;

c) diluting the aqueous solution with water to a solids content of 5 to 25 weight percent;

d) cooling the diluted aqueous solution to precipitate and separate lipophilic components from the diluted aqueous solution;

e) treating the aqueous solution from step (d) with ammonium sulfate and and then extracting the aqueous solution with methylethylketone, acetone, or a mixture of methylethylketone and acetone;

f) diluting the extract from step (e) with water and alcohol to form an aqueous alcohol solution;

g) treating the aqueous alcohol solution with a lead compound or an insoluble polyamide;

h) extracting the treated aqueous alcohol solution with an aliphatic or cycloaliphatic solvent having a boiling point of about 60.degree.-100.degree. C. to further remove the alkylphenol compounds; and

i) recovering a dry extract.

6. The method of claim 5 wherein the solids content of step (c) is about 15 to 20% by weight.

7. A method of preparing an extract from Ginkgo biloba leaves, containing 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent of ginkgolides selected from ginkgolide A, B, C and J or mixtures thereof, 2.0 to 4.0 weight percent bilobalide, less than 10 ppm alkylphenol compounds and less than 10 weight percent proanthocyanidins comprising the steps of:

(a) extracting fresh or dried green leaves of Ginkgo biloba at a temperature of approximately 40.degree. to 100.degree. C. with an organic solvent selected from the group consisting of aqueous acetone, an aqueous alkanol of 1 to 3 C-atoms and anhydrous methanol,

(b) distilling the extract from step (a) to remove the organic solvent to a maximum content of 10 weight percent to form a concentrated aqueous solution,

(c) diluting the concentrated aqueous solution with water to a solids content of 5 to 25 weight percent and then cooling the diluted aqueous solution to a temperature below 25.degree. C. to precipitate and separate the lipophilic components from the diluted aqueous solution,

(d) adding ammonium sulfate to the aqueous solution from step (c) to a concentration of 30 weight percent and extracting said solution with methylethylketone or a mixture containing methylethylketone and acetone in a ratio from about 9:1 to 4:6,

(e) concentrating the extract from step (d) to a solids content of 50 to 70% and then diluting with water and ethanol to form an aqueous alcohol solution containing about 50 weight percent of water and about 50 weight percent of ethanol with a solids content of about 10 weight percent,

(f) adding an aqueous solution of a lead salt or an aqueous suspension of lead hydroxide to the aqueous alcohol solution of step (e) until a change in color from brown to umber takes place and precipitate is formed and separated,

(g) extracting the aqueous alcohol solution from step (f) with an aliphatic or cycloaliphatic solvent having a boiling point of about 60.degree. to 100.degree. C. to further remove alkylphenol compounds,

(h) concentrating the aqueous alcohol solution from step (g) under reduced pressure to a maximum ethanol content of about 5% and then adding ammonium sulfate up to about 20 weight percent,

(i) extracting the aqueous alcohol solution from step (h) with a mixture of methylethylketone and ethanol in a ratio from about 8:2 to 5:5 to form an organic phase extract,

(j) concentrating the organic phase extract to a solids content of 50 to 70 weight percent, and

(k) drying the resultant concentrate from step (j) under reduced pressure at a maximum temperature of 60.degree. to 80.degree. C. to form a dry extract with a water content of less than 5%.

8. The method of claim 7 wherein the prepared extract contains about 22 to 26% by weight flavone glycosides.

9. The method of claim 7 wherein the prepared extract contains less than 1 ppm alkylphenol compounds.

10. The method of claim 7 wherein the extract of step (b) contains a maximum of 5 weight percent organic solvent.

11. The method of claim 7 wherein the solids content of step (c) is about 15 to 20% by weight.

12. The method of claim 7 wherein the extract of step (c) is cooled to about 10.degree. to 12.degree. C.

13. The method of claim 7 wherein the methylethylketone and acetone mixture of step (d), step (i), or step (d) and step (i) is in a ratio of 6 to 4.

14. The method of claim 7 wherein the lead salt in step (f) is selected from the group consisting of lead acetate, lead hydroxide acetate and lead nitrate.

15. The method of claim 7 wherein lead hydroxide acetate is added to the aqueous solution of step (f).

16. A pharmaceutical composition comprising a Ginkgo biloba extract prepared according to the process of claim 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 in a pharmaceutical carrier wherein the extract concentration is sufficient to alleviate circulatory disorders.

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DOCUMENT-IDENTIFIER: US 5322688 A

TITLE: Method of preparation of an extract from Ginkgo biloba leaves and pharmaceuticals containing the extract

DATE-ISSUED: June 21, 1994

INVENTOR-INFORMATION:

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PARENT-CASE:
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US-CL-CURRENT: 424/752; 514/27

FIELD-OF-SEARCH: 424/195.1, 514/27

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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0324197	July 1989	EP	424/195.1

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ART-UNIT: 185

PRIMARY-EXAMINER: Wityshyn; Michael G.

ASSISTANT-EXAMINER: Gitomer; Ralph G.

ABSTRACT:

The invention relates to a method of preparation of an improved extract from Ginkgo biloba leaves and to pharmaceuticals containing the extract.

12 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1 The invention relates to a method of preparation of an improved extract from Ginkgo biloba leaves and to pharmaceuticals containing the extract.

2 Extracts from the leaves of Ginkgo biloba have been used for a long time for the therapy of peripheral and cerebral arterial circulatory disturbances. Methods of preparation of Ginkgo biloba extracts with a greatly enriched content of flavone glycosides as the active components are known; see DE-B 17 67 098 and DE-B 21 17 429. These extracts are also referred to as Ginkgo biloba monoextracts.

3 EP-A 0 324 197 describes a method of preparation of an extract from Ginkgo biloba leaves in which an aqueous solution of a lower alcohol or ketone, obtained after extraction of the leaves, is concentrated in the presence of kieselguhr. The resultant aqueous suspension is filtered through kieselguhr, the fitrate is extracted with butanone and the extract is freed from the solvent.

4 EP-A 330 567 relates to a method of preparation of an extract from Ginkgo biloba leaves in which the crushed leaves extracted with an aqueous ketone compound. This extract is concentrated until biflavones and hydrophobic compounds precipitate. After filtration the aqueous concentrate is rendered alkaline, whereby the proanthocyanidins precipitate. After separation of the precipitate and acidification of the filtrate, a liquid-liquid-extraction is carried out with a C.sub.4-6 -ketone compound in the presence of ammonium sulfate. The extract is obtained after stripping of the ketone compound.

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7 Besides the compounds mentioned above, Ginkgo biloba leaves also contain so-called ginkgolic acids (anacardic acids). These compounds are 6-alkylsalicylic acids with n-C.sub.13 - to n-C.sub.19 -alkyl groups with 0 to 3 double bonds; see J. L. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961 and Analytic. Chem., Vol. 40 (1968), 739-743.

8 "Ginkgol", a phenol substituted with the corresponding alkyl group, can be obtained either biogenetically by decarboxylation of the ginkgolic acids or during the technical processing of the Ginkgo biloba leaves; see Kawamura, Japan, J. Chem., Vol. 3 (1928), 91-93.

9 The ginkgolic acids and ginkgols in Ginkgo biloba are accompanied by corresponding derivatives with a further phenolic hydroxyl group in 4-position, the 6-alkylresorcylic acids or 5-alkylresorcins; see J. Gellermann et al., Phytochemistry, Vol. 15 (1976), 1959-1961. These resorcin derivatives are responsible for the toxic effects and especially for the strong allergies and contact dermatitis caused by toxicodendron plants; see G. A. Hill et al., J. Am. Chem. Soc., Vol. 56 (1934), 2736-2738.

10 Cases of strong allergic reactions after contact with Ginkgo fruits are known; see W. F. Sowers et al., Arch. Dermatol., Vol. 91 (1965), 452-456, and T. Nakamura, Contact Dermatitis, Vol. 12 (1985), 281-282. Serious mucosal disturbances after eating Ginkgo fruits have been described; see L. E. Becker and G. B. Skipworth, J. Am. Med. Assoc., Vol. 231 (1975), 1162-1163. Allergic skin reactions also occur occasionally on collecting or handling Ginkgo leaves.

11 The significance of allergies caused by alkylphenol compounds from anacardiaceae and ginkgoaceae is evident from the development of substances and methods of desensitisation described in patent literature (see U.S. Pat. No. 4 428 965) against the allergies caused by alkylphenol compounds.

12 Commercial extracts from Ginkgo biloba leaves contain between 50 and 10,000 ppm ginkgolic acids.

13 The extracts from Ginkgo biloba leaves prepared by the known methods in DE-B 17 67 098 and DE-B 21 17 429 are substantially free of alkylphenol compounds because the lipophilic components of the extract are removed by a liquid-liquid-extraction of the aqueous acetone extract with a substantially water-immiscible lipophilic solvent, e.g. with a chlorinated aliphatic lower hydrocarbon such as carbon tetrachloride. However, in this step, the therapeutically valuable ginkgolides and the bilobalide are also considerably reduced so that their content in the final product in Example 1 of DE-B 21 17 429 is a maximum of 0.5% in the case of ginkgolides A, B, C and J in total and approximately 0.3% in the case of bilobalide. The quantity of flavone glycosides, however, is greatly increased during this step, namely from 3 to 4% in the crude extract to approximately 24% in the final product.

14 The object of the present invention is to provide a method of preparation of the extract from Ginkgo biloba leaves which is substantially free of alkylphenol compounds and which has a high content of flavone glycosides, ginkgolides and bilobalide. The method of the present invention should, in contrast to the known methods in DE-B 17 67 098 and DE-B 21 17 429, succeed in removing the alkylphenol compounds without the use of chlorinated aliphatic hydrocarbons. The use of chlorinated hydrocarbons in technical processes is very problematic because of the occupational medical risks, the potential danger of these

compounds to the environment and the possibility of undesirable residues in pharmaceuticals.

15 A further advantage of the method of the present invention compared to the method in DE-B 21 17 429 is that no lead compounds are used in the removal of the undesirable polyphenol compounds with tanning properties (proanthocyanidins). Compounds of lead are most undesirable because of the health risks for the people involved, and over and above that, the costs involved for their proper disposal are considerable.

16 It is a further object of the invention to provide pharmaceuticals which contain this Ginkgo biloba extract with a high content of flavone glycosides, ginkgolides and bilobalide and where there is substantially no danger of allergic reactions, precisely because of the removal of the alkylphenol compounds.

17 The invention therefore relates to a method of preparation of this Ginkgo biloba extract from Ginkgo biloba leaves which comprises the steps described in claims 1-4. In contrast to the method of separating the lipophilic components described in DE-B 17 67 098, the aqueous alcohol or aqueous acetone crude extract is not directly subjected to liquid-liquid-extraction with a chlorinated aliphatic hydrocarbon, but rather most of the lipophilic components, which precipitate on distillation of the organic solvent components and dilution with water to a maximum content of 10 weight percent, preferably 5 weight percent, are separated by filtration. The alkylphenol compounds, the chlorophyll, the fatty acid derivatives and the biflavones precipitate due to their low solubility in water and can be separated by filtration. Under these conditions, the desired components of the Ginkgo biloba extract remain dissolved.

18 Subsequently, the methylethylketone or methylethylketone/acetone-extracts are prepared according to DE-B 17 67 098 and DE-B 21 17 429. In contrast to the method in DE-B 21 17 429, however, a lead compound or a polyamide is not used to reduce the content of proanthocyanidins to less than 10%, but rather a distribution of the butanone extract is carried out between water and a water-immiscible C.sub.4-5 -alkanol, whereby the proanthocyanidins remain in the water phase.

19 In a preferred embodiment the extraction with methylethylketone or methylethylketone/acetone is directly replaced by extracting the aqueous extract solution freed from the lipophilic components with a water-immiscible alkanol of 4 or 5 C-atoms. For economic reasons n-butanol is preferred. 10 to 30 weight percent of sodium chloride or ammonium sulfate can be added to the aqueous extract solution. The alkylphenol compounds are reduced further to a content of less than 10 ppm in a subsequent decreasing step by removing the solvent from the butanol or pentanol extract by distillation, preparing a solution with 5 to 20 weight percent solids content in 20 to 60 weight percent of aqueous ethanol and subjecting this solution to a multistep liquid-liquid-extraction with an aliphatic hydrocarbon with a boiling point of 60.degree. to 100.degree. C.

20 In addition, the invention relates to pharmaceuticals which according to claim 5 are characterized by a content of Ginkgo biloba extract.

21 In pharmacological experimental models, the extract prepared according to the present invention has radical scavenging properties and properties which stimulate the circulation of blood, prevent ischemic disorders and inhibit platelet aggregations.

22 The Ginkgo biloba extract of the invention can be processed in the usual way for the preparation of pharmaceuticals e.g. to solutions, coated tablets, tablets or injection preparations. The pharmaceuticals in the invention are used for the treatment of peripheral and cerebral arterial circulatory disturbances.

23 The examples illustrate the invention. Parts and percentage data refer to weight unless otherwise stated.

DETAILED DESCRIPTION:

1 EXAMPLE 1

2 100 kg of dry Ginkgo biloba leaves are crushed in a mill to a particle size of less than 4 mm. After adding 750 kg of 60 weight percent aqueous acetone the mixture is stirred intensively for 30 minutes at a temperature of 57.degree. to 59.degree. C. The solid residue is separated by filtration or centrifugation and subjected to a second extraction under the same conditions. The extracts from the first and second extraction steps are combined. The ginkgolic acid content (based on the dry extract) equals approximately 13,000 ppm. The resultant extract is concentrated under reduced pressure to a solids content of 30 to 40% and a maximum of approximately 5 weight percent acetone. By adding water, the concentrate is diluted to double volume and, while being stirred, left to cool to approximately 12.degree. C. A precipitate forms which contains most of the ginkgolic acids, that is, the alkylphenol compounds, present in the leaves. After one hour at this temperature, the resultant precipitate is separated by centrifugation and discarded.

3 The ginkgolic acid content in the resultant aqueous supernatant (based on the dry extract) equals approximately 320 ppm.

4 30 parts of ammonium sulfate are added to 100 parts of the aqueous solution. The mixture is stirred. After the ammonium sulfate has dissolved, a liquid-liquid-extraction is carried out twice with a mixture of methylethylketone and acetone in a ratio of 6:4 to 1:1, whereby the organic solvent added is equivalent to half the volume of the aqueous solution and, after intensive stirring and pumping, the organic upper phase formed on completion of the mixing process is removed.

5 The methylethylketone acetone solution is then concentrated under reduced pressure to a solids content of 50 to 70%. This concentrate is diluted with water to a solids content of 10%. This substantially aqueous extract solution is stirred three times, each time with half of its volume of water-saturated n-butanol. The combined butanol phases are concentrated under reduced pressure to a solids content of at least 50%. To remove the n-butanol from the highly concentrated extract by azeotropic distillation, water, preferably, is added. The resultant aqueous concentrate is diluted with water and ethanol so that a solution with 10 weight percent dry extract in 30 weight percent aqueous ethanol is obtained.

6 To reduce the alkylphenol compounds to a residual content of less than 10 ppm, this solution is stirred at least three times at room temperature, each time with 1/3 of its volume of n-heptane.

7 The water phase is concentrated under reduced pressure to a solids content of at least 50% and dried at a maximum product temperature of approximately 60.degree. to 80.degree. C. to a dry extract with a water content of less than 5%.

8 From 100 kg of Ginkgo leaves, 2.7 kg of Ginkgo biloba extract with a content of 24.8 weight percent flavone glycosides, 3.2% ginkgolides, 2.9% bilobalide, approximately 5% proanthocyanidins and less than 1 ppm alkylphenol compounds are obtained.

9 EXAMPLE 2

10 The aqueous extract solution obtained in Example 1, following separation by centrifugation of the precipitate consisting predominantly of lipophilic components, is stirred three times, each time with half of its volume of butan-2-ol (sec. butylalcohol).

11 The resultant butan-2-ol solution is evaporated under reduced pressure until a concentrate with at least 50% solids content is obtained. Preferably water is

added to remove the butanol from the highly concentrated extract by azeotropic distillation. Following dilution with water and ethanol to a solids content of approx. 10% and approx. 30 percent by weight ethanol in the solution, the solution is stirred three times, each time with 1/3 of its volume of cyclohexane.

12 The water phase is concentrated under reduced pressure to a solids content of at least 50% and dried at a maximum temperature of 60.degree. to 80.degree. C. to a dry extract with a water content of less than 5%.

13 From 100 kg of Ginkgo leaves, 2.9 kg of Ginkgo biloba extract with a content of 25.3% flavone glycosides, 3.4% ginkgolides, 3.1% bilobalide, approximately 4.2% proanthocyanidins and less than 1 ppm alkylphenol compounds are obtained.

14 EXAMPLE 3

15 Solution for oral administration:

100 ml solution contains:		
Ginkgo biloba extract	4.0	g
ethanol	50.0	g
demineralised water to	100.0	ml

16 EXAMPLE 4

17 Coated tablets:

1 tablet contains:	
Ginkgo biloba extract	40.00 mg
microcrystalline cellulose	100.00 mg
lactose	80.00 mg
colloidal silicic acid	25.00 mg
talcum (in core)	4.50 mg
magnesium stearate	0.50 mg
hydroxypropyl methylcellulose	12.00 mg
ferric oxide pigment	0.10 mg
talcum (in coat)	0.50 mg
weight of a coated tablet approx.	262.60 mg

CLAIMS:

I claim:

1. A method of preparing an extract from the leaves of Ginkgo biloba which is substantially free of alkylphenol compounds and having a high content of flavone glycosides and comprising substantially all of the ginkgolides and bilobalide originally present in the leaves, the method comprising the steps of

a) extracting the leaves with an organic solvent selected from the group

consisting of aqueous acetone, an aqueous alkanol having one to three carbon atoms and anhydrous methanol;

b) separating most of the organic solvent from the extract of step (a) by evaporation or distillation, optionally at reduced pressure, to form an aqueous solution;

c) diluting the aqueous solution with water to a solids content of 5 to 25 weight percent;

d) cooling the diluted aqueous solution to precipitate and remove the water-insoluble lipophilic components from the diluted aqueous solution;

e) treating the aqueous solution from step (d) with 10-30% ammonium sulfate then extracting the aqueous solution with a solvent selected from the group consisting of methylethylketone and a mixture of methylethylketone and acetone;

f) extracting the extract from step (e) with butanol or pentanol;

g) diluting the butanol or pentanol extract from step (f) with water and alcohol to form an aqueous alcohol solution;

h) extracting the aqueous alcohol solution with an aliphatic or cycloaliphatic solvent having a boiling point of about 60.degree.-100.degree. C. to further remove the alkylphenol compounds; and

i) concentrating the aqueous extract solution resultant from step (h) under reduced pressure and drying the resultant concentrate at a maximum temperature of 60.degree. to 80.degree. C. to form a dry extract with a water content of less than 5 weight percent.

2. A method of preparing an extract from Ginkgo biloba leaves, containing 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent of ginkgolides A, B, C and J, 2.0 to 4.0 weight percent bilobalide, less than 10 ppm alkylphenol compounds and less than 10 weight percent proanthocyanidins comprising the steps of:

(a) extracting fresh or dried green leaves of Ginkgo biloba at a temperature of approximately 40.degree. to 100.degree. C. with an organic solvent selected from the group consisting of aqueous acetone, an aqueous alkanol of 1 to 3C-atoms and anhydrous methanol;

(b) vacuum distilling the extract from step (a) to remove the organic solvent to a maximum content of 10 weight percent to form a concentrated aqueous solution;

(c) diluting the concentrated aqueous solution with water to a solids content of 5 to 25 weight percent then cooling the diluted aqueous solution to a temperature below 25.degree. C. to precipitate and remove the water-insoluble lipophilic components from the diluted aqueous solution;

(d) adding ammonium sulfate to the aqueous solution from step (c) to a concentration of 30 weight percent and extracting said solution with a solvent selected from the group consisting of methylethylketone and a mixture of methylethylketone and acetone in a ratio from about 9:1 to 4:6;

(e) concentrating the extract from step (d) to a solids content of 50 to 70% then diluting with water to a solids content of about 10 weight percent;

(f) extracting the aqueous concentrate from step (e) with water-immiscible C.sub.4 or C.sub.5 alkanol to form alkanol layers;

(g) concentrating the alkanol layers to a solids content of 50 to 70 weight percent;

(h) diluting the concentrate of step (g) with water and ethanol to form a

solution having 5 to 20 weight percent dry extract in 20 to 60 weight percent aqueous ethanol;

(i) extracting the aqueous alcohol solution from step (h) with an aliphatic or cycloaliphatic solvent having a boiling point of about 60.degree. to 100.degree. C. to further remove alkylphenol compounds;

(j) concentrating the aqueous extract solution resultant from step (i) under reduced pressure and drying the resultant concentrate at a maximum temperature of 60.degree. to 80.degree. C. to form a dry extract with a water content of less than 5 weight percent.

3. The method of claim 2 wherein the dry extract contains about 22 to 26% by weight flavone glycosides.

4. The method of claim 2 wherein the dry extract contains less than 1 ppm alkylphenol compounds.

5. The method of claim 2 wherein the concentrated aqueous solution of step (b) contains a maximum of 5 weight percent organic solvent.

6. The method of claim 2 wherein the solids content of step (c) is about 15 to 20% by weight.

7. The method of claim 2 wherein the diluted aqueous solution of step (c) is cooled to about 10.degree. to 12.degree. C.

8. The method of claim 2 wherein the methylethylketone and acetone mixture of step (d) is in a ratio of 6 to 4.

9. The method of claim 2 wherein the alkanol of step (f) is n-butanol or pentanol.

10. A method of preparing an extract from Ginkgo biloba leaves, containing 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent of ginkogolides A, B, C and J, 2.0 to 4.0 weight percent bilobalide, less than 10 ppm alkylphenol compounds and less than 10 weight percent proanthocyanidins comprising the steps of:

(a) extracting fresh or dried green leaves of Ginkgo biloba at a temperature of approximately 40.degree. to 100.degree. C. with an organic solvent selected from the group consisting of aqueous acetone, an aqueous alkanol of 1 to 3C-atoms and anhydrous methanol;

(b) vacuum distilling the extract from step (a) to remove the organic solvent to a maximum content of 10 weight percent to form a concentrated aqueous solution;

(c) diluting the concentrated aqueous solution with water to a solids content of 5 to 25 weight percent, and then cooling the diluted aqueous solution to a temperature below 25.degree. C. to precipitate and remove the water-insoluble lipophilic components from the diluted aqueous solution;

(d) extracting the aqueous extract solution from step (c) with a water-immiscible C.sub.4 to C.sub.5 alkanol layer said aqueous solution optionally containing 10 to 30 weight percent of sodium chloride or ammonium sulfate;

(e) concentrating the alkanol layer to a solids content of 50 to 70 weight percent;

(f) diluting the concentrate of step (e) with water and ethanol to form a solution having 5 to 20 weight percent dry extract in 20 to 60 weight percent aqueous ethanol;

(g) extracting the aqueous ethanol solution from step (f) with an aliphatic or

cycloaliphatic solvent having a boiling point of about 60.degree. C. to 100.degree. C. to further remove alkylphenol compounds;

(h) concentrating the aqueous extract solution from step (g) under reduced pressure;

(i) drying the resultant concentrate from step (h) at a maximum temperature of 60.degree. C. to 80.degree. C. to form a dry extract with a water content of less than 5 weight percent.

11. The method of claim 10 wherein the alkanol of step (d) is n-butanol or pentanol.

12. A pharmaceutical composition comprising a Ginkgo biloba extract prepared according to the process of any one of claims 1, 2 and 10 in a pharmaceutical carrier.

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TITLE: Treatment or prevention of PAF Acether-induced maladies

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ART-UNIT: 123

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ABSTRACT:

The present invention relates to the treatment of PAF Acether-induced maladies. The treatment comprises the administration of a ginkgolide or a ginkgolide derivative. Ginkgolide B has been found to be the most effective. The ginkgolides may be introduced orally, intraveneously or by spray inhalation.

12 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

BRIEF SUMMARY:

1 The present invention relates to the treatment or prevention of PAF Acether-induced maladies. The treatment comprises the administration of an effective amount of one or more ginkgolides or derivatives thereof, alone or in admixture with a pharmaceutically acceptable diluent or carrier.

2 PAF Acether (Platelet Activating Factor) is a known phospholipid which can cause maladies in animals, including humans. As reported by Benveniste et al., the PAF Acether causes aggregation of platelets and provokes the release of their vasoactive amines. As also reported by Benveniste, it also stimulates neutrophils to aggregate and release their proinflammatory content, see J. BENVENISTE, C. BOULLET, C. BRINK and C. LABAT, Brit. J. Pharmacol, The action of PAF Acether on guinea-pig isolated heart preparation, 80: 81-3 (1983).

3 It is commonly understood that a PAF Acether release is induced in animals, including humans, upon the happening of various kinds of shocks such as anaphylactic shocks, burns, septic shocks, irradiation shocks and traumae. There is a strong release of PAF-Acether in the hours following the shock, which leads thereafter to a suppression of immunitory reactions due to the exhaust of the defensive means of the organism. While many compounds have been tried for the treatment or prevention of PAF Acether-induced maladies, the rate of effectiveness has not been very great. These treatments are discussed more fully in the examples infra.

4 The applicant has now discovered that PAF Acether-induced maladies can be effectively treated by the administration of a ginkgolide or a ginkgolide derivative. The ginkgolides are in many cases surprisingly more effective in treatment of PAF Acether-induced maladies than the compounds which are presently used for the treatment of PAF Acether-induced maladies. Of equal or greater advantage is the highly selective nature of the ginkgolides. While they are highly effective against platelet aggregation caused by PAF Acether, they exhibit virtually no inhibition against platelet aggregation caused by other factors. In particular, they do not interfere with aggregations induced by ADP, thrombine, collagen, adrenaline, histamine, LTB.sub.4 -, and serotonin. The applicant's discovery of this highly selective nature of inhibition of PAF Acether-induced platelet aggregation by the ginkgolides is most important.

5 Ginkgolides have been known for many years. They have been found in Ginkgo Biloba extracts but, to date, no therapeutical activity has been found for this group of compounds. These compounds may be obtained as described by KOHI NAKANISHI, Pure and Applied Chemistry, 14 (1967) 89. Their structures are also

described by MARUYAMA and Al., Tetrahedron Letters (1967) 299, 303.

6 The ginkgolides are derived from the gymnospermous tree Ginkgo Biloba. Commmonly available ginkgolides include Ginkgolide A, Ginkgolide B, Ginkgolide C, and Ginkgolide M. Each of these has been found to be effective in treating PAF Acether-induced maladies. Of these four, Ginkgolide B has been found to be the most effective.

7 Derivatives of the ginkgolides are also known. Common derivatives include the mono-acetate, the tri-acetate, the tetrahydro and the acetyl, see K. OKABE, K. YAMADA, S. YAMAMURA, and S. TAKADA, Ginkgolides, J. Chem. Soc. (C), 1967, 2201-2206.

8 In the treatment of PAF Acether-induced maladies, the ginkgolides can suitably be administered orally, intravenously (including by perfusion), or by spray inhalation at doses from 1 to 50 mg/kg. In addition to use in the treatment of PAF Acether-induced maladies, the ginkgolides can also be used to prevent the onset of PAF Acether platelet aggregation in high risk situations.

9 The antagonist action of Ginkgolides administration against the platelets aggregation incuded by PAF-Acether brings a correlative shortening of the post-trauma period during which the immunitory means of the organism are lowered or suppressed.

DETAILED DESCRIPTION:

1 These and other advantages of the present invention may be more fully understood by the discussion which follows concerning the effectiveness of the compounds in treatment of PAF Acether-induced maladies as compared to literature-reported compounds for the same treatment.

2 I Binding Determinations

3 The competition of the alternative binding of either .sup.3 H-PAF Acether or one of the Ginkgolides was evidenced by radioactive determinations using a rabbit membrane platelet preparation. (100% control=.sup.3 H-PAF Acether alone). At 10.sup.-4 M, Ginkgolide M reduced the binding by 79%. At 10.sup.-5 M, Ginkgolides A and C reduced the binding respectively by 67 and 83%. At 10.sup.-6 M, Ginkgolide B reduced the binding by 94%.

4 IC.sub.50 determined in these conditions for Ginkgolide B was equal to 5.75 10.sup.-7 M.

5 For testing the specificity of action of these compounds, on PAF-Acether receptor the binding of the Ginkgolides was also checked on other types of receptors: H.sub.1, H.sub.2, 5-HT, 5HT.sub.2, .alpha..sub.1, .alpha..sub.2, .beta..sub.1, .beta..sub.2, etc . . . : no noticeable binding was seen, demonstrating their specificity of action.

6 II Inhibition of Platelets Aggregation (PA)

7 1-Rabbit PA

8 1.1. In vitro study:

9 The Inhibition of PAF Acether-induced PA has been studied on rabbit plasma-rich platelets (PRP). Two doses of PAF Acether were used (2.5 nM and 5 nM). The tested compound was added to the medium 3 minutes before PAF Acether and aggregation was followed by monitoring changes in electrical impedance (Whole-Blood aggregometer-chrono-Log); kadsurenone was simultaneously tested as reference compound and the IC.sub.50 molar doses of Ginkgolides A, B, C and M and of kadsurenone on PAF Acether-induced rabbit PA (PRP) have been calculated.

10 The results obtained are presented in the following table (average values for 10 assays for each dose) and FIG. 1:

	For PAF Acether doses (in nM) IC.sub.50 doses of (M)	
	2.5	5
Ginkgolide A	8.32 10.sup.-6	1.32 10.sup.-5
Ginkgolide B	1.88 10.sup.-6	3.20 10.sup.-6
Ginkgolide C	1.53 10.sup.-5	2.54 10.sup.-5
Ginkgolide M	1.22 10.sup.-4	2.02 10.sup.-5
Kadsurenone	9.94 10.sup.-6	1.40 10.sup.-5

11 Ginkgolides A, B, C and M were totally ineffective against aggregation induced by other known aggregants such as: ADP, thrombine, calcium ionophore A 23187, collagen and adrenaline.

12 This result shows the high specificity of action of Ginkgolides.

13 FIG. 2 present the dose/activity relationship obtained with Ginkgolide B.

14 1.2. Ex vivo study:

15 Drugs were administered per os (2,5 and 10 mg/kg) or by IV route (0.5, 1, 2 mg/kg) and blood samples were taken at different times to test platelet aggregation with PAF-Acether (2.5 and 5 nm) (see following table wherein the figures appearing are the ratio to the control with the solvent used, i.e. DMSO).

INHIBITION OF PAF ACETHER-INDUCED RABBIT PA BY GINKGOLIDE B (% of control)(*)

Administration Mode (mg/kg)	PAF Acether 2.5 nM 0.5 h	1 h	2 h	5.0 nM 0.5 h	1 h	2 h
PER OS						
2	23	37	54	34.5	47.8	71.2
5	0	7	10	6	11.4	27.1
10	0	0	0	0	0	0
IV						
0.5	0	7.4	11.2	0	9.4	14.5
1	0	0	4.1	0	1.1	7.2
2	0	0	0	0	0	0

(*) average values for one batch of 5 assays.

16 2-Human PA

17 2.1. In vitro study

18 2.1.1. PRP

19 The inhibition of PAF Acether-induced PA has been studied on human PRP. The same protocol was used as for rabbit hereinabove.

IC Doses (M) of	PAF Acether Dose (nM) 100
Ginkgolide A	9 10.sup.-5
Ginkgolide B	5.6 10.sup.-5
Ginkgolide C	1.5 10.sup.-4
Kadsurenone	1.3 10.sup.-4

20 All ginkgolides were more effective than kadsurenone.

21 FIG. 3 shows the comparison of the inhibition obtained with the most potent Ginkgolide (GK B) and the reference product (kadsurenone).

22 2.1.2. Washed Platelets

23 The inhibition of PAF Acether-induced PA was also experimented using human washed platelets on Ginkgolides A, B, and C.

24 Best figure for IC.sub.50 was obtained with Ginkgolide B (GK B) (average values of 5 assays) with 2.5 10.sup.-5 M.

25 FIG. 4 shows the dose/activity relationship.

26 2.2. Ex vivo study (Ginkgolides A, B and C)

27 The same protocol described hereinabove for rabbit was used in man with only one dose of each Ginkgolide (IV 25 mg) and two blood takes (at 0.5 h and 1 h). Results are given in the following table:

Time before blood take (hours)	PAF Acether (100 nM) 0.5	1.0
% of aggregation with respect to control	GK A 0	5.7
	GK B 0	3.4
	GK C 0	10.6

28 3-Opto electronic follow-up of platelet wessel wall interaction

29 In this experimental study, 20 guinea-pigs were used. The technical details concerning the operative procedure, the induction of focal deendothelialization, and the methodology of the electronically controlled microprojection procedures have been previously described in detail (Bourgain, R., and Six, F. (1974): a continuous registration method in experimental arterial thrombosis in the rat. Thromb. Reg. 4: 599-607). Briefly summarized, this method consists in the dissection over a distance of 2 to 3 mm of a branch of the mesenteric artery following gently extraction of a loop of small intestine. By transillumination,

the image of this arterial segment is projected onto a set of 30 light-depending resistances (LDR) arranged in two columns of 15 elements each. An LDR element has the property to change its internal resistance as a function of a variation of light intensity. Each LDR is connected into its own Wheatstone bridge, and the changes in light intensity due to thrombus formation will be recorded as variations in electrical potentials and registered as such via a multiplexing device.

30 In the present investigation, several discriminating parameters are recorded: (a) the t.sub.1 interval or the lag period (this is the time interval between the beginning of the PAF Acether superfusion and the first signs of thrombus formation; (b) the t.sub.d interval is the duration of the thrombotic phenomenon; (c) the O(t) curve indicates the number of LDR elements covered by the thrombus image; (d) the D(t) curve represents during each 30.times.1/9.times.10.sup.-2 sec interval the greatest potential deviation on one of the LDR elements covered by the thrombus image; (e) the T(t) curve represents the summation of the potentials registered on the LDR elements during a time interval of 30.times.1/9.times.10.sup.-2 sec the TTV(t) curve results from the integration of the T(t) values in function of time; (f) m.sub.D, m.sub.T, and m.sub.O correspond to the maximal values registered on, respectively, the D(t), T(t), and O(t) curves; (g) TTV is the maximal value of the TTV(t) curve, whereas TVM is the value of this curve up to the point where m.sub.T is reached.

31 In a first series of experiments (10 animals) the effect of local superfusion with PAF Acether (10.sup.-4 M) is investigated.

32 In a second series of experiments (10 animals) the effects of Ginkgolide B and of kadsurenone have been compared preventively and curatively.

33 PAF Acether superfusion induced a large and occlusive thrombus. Only prostacycline superfusion in the same time than PAF Acether superfusion might partially inhibit this phenomenon. Furthermore, to date, no drug was known to have a curative action against a pre-formed thrombus while Ginkgolide B or kadsurenone, when superfused with PAF Acether, no thrombus appeared demonstrating the efficiency of these agents.

34 Surprisingly, as shown in the following figures, Ginkgolide B and kadsurenone were able to disaggregate such pre-formed thrombus but with a strong advantage for Ginkgolide if time is taken into consideration (about 4.30 minutes for disappearance of thrombus with kadsurenone against 2.30 minutes for Ginkgolide B).

35 FIG. 5 shows the results obtained after the third superfusion of PAF Acether in the guinea-pig mesenteric artery.

36 III Isolated organs

37 3.1. Contraction of superfused lung parenchymal strip induced by PAF Acether

38 The antagonistic effect of preventive superfusion of 30 .mu.g/ml of each of the Ginkgolides against PAF Acether (3 ng)-induced contraction of guinea-pig lung parenchymal strip (Ref. Giovanni CAMUSSI, Giuseppe MONTRUCCHIO, Camillo ANTRO, Federico BUSSOLINO, Ciro TETTA and Giorgio EMANUELLI, "Platelet-activating Factor-mediated Contraction of Rabbit Lung Strips: Pharmacologic Modulation, Immunopharmacology 6: 87-96 (1983) has been determined using an isometric transducer connected to a Gould recorder.

39 The results presented in the following table showed a very significant inhibition of the PAF Acether-induced contraction.

Contraction induced by PAF Acether

	(3 ng) (Arbitrary Units)*
Control	39.82 .+-. 8.24
DMSO (solvent)	37.90 .+-. 6.24
Ginkgolide A (30 .mu.g/ml)	10.30 .+-. 2.33 (-72.8%)**
Ginkgolide B (30 .mu.g/ml)	8.05 .+-. 2.16 (-78.8%)**
Ginkgolide C (30 .mu.g/ml)	11.08 .+-. 2.42 (-70.8%)**

*average value of 10 determinations
**p < 0.001

40 The Ginkgolides were without significant effect on histamine-, LTB.sub.4 - or LTD.sub.4 -induced contractions.

41 3.2. Isolated Rat Portal Vein

42 In vitro experiments were performed on isolated longitudinal strips of rat portal vein maintained in Krebs-Henseleit medium at 37.degree. C. under an oxygen-rich gaseous circulation (95% O.sub.2 -5% CO.sub.2).

43 The preparations were allowed to equilibrate for 1 hour under isotonic conditions with 500 mg tension and rinced every 15 minutes. When myogenic activity was established, PAF Acether (10.sup.-7 M) is added. It induced an increase of the basal tonus and frequency of the myogenic activity.

44 Ginkgolide B (tested from 1 to 100 g/ml) added 30 minutes before PAF Acether exerted a dose related antagonistic effect on the increase of the basal tonus with IC.sub.50 =6.8 .mu.g/ml. The drug was without effect on the myogenic activity showing that it has no calcium-antagonistic activity.

45 3.3. Isolated Langendorff heart

46 The deletereous effect of PAF Acether on Isolated Langendorff heart has been previously described by J. BENVENISTE, C. BOULLET, C. BRINK and C. LABAT, Brit. J. Pharmacol, The action of PAF Acether on guinea-pig isolated heart preparation, 80: 81-3 (1983).

47 PAF Acether (100 pM) induced a decline in contractile strength (-30%) and a decrease of coronary flow (-50%).

48 Ginkgolide B (2.10.sup.-4 M) totally inhibited these phenomena. This effect was dose-dependent (disappearance of protective activity when dose .ltoreq.10.sup.-7 M).

49 IV Antianaphylactic action and protective effect against fluid escape and shock

50 4.1. Cutaneous vascular hyperpermeability induced by PAF Acether in the rat

51 Method: Intradermal injection of 25 ng of PAF Acether in the dorsal region of the rat with simultaneous IV injection of Evans blue dye (60 mg/kg/5 ml) induces vascular hyperpermeability and wheals formation. (for details see Basran GS, Page CP, Paul W and Morley J-Cromoglycate inhibits responses to platelet-activating factor (PAF Acether) in man: an alternative mode of action for DSCG in asthma-Eur. J. Pharma. (1983) 86 143-144).

52 These wheals were measured (surface in mm.sup.2) at the inner side of the skin 30 mn after the injections and were taken off, put in 4 ml of formamide, incubated at 65.degree. C. during 24 h in order to extract the dye and measure

their coloration (optical density at 620 nm).

53 Groups of 6 Sprague-Dowley rats weighing 200 g were used and each rat received intradermal injections in two sites of the back. The experiment was performed in comparison with a reference compound, D 600 (methoxy verapamil), a calcium-blocker agent.

54 Treatments: Tested compounds were administered by IV route injected once simultaneously with the dye.

55 The results obtained are reported in the following table:

VASCULAR HYPERPERMEABILITY INDUCED BY PAF ACETHER IN THE RAT
($p < 0.001$ for all experiments)

		PAF Acether induced wheal			
	Dose mg/kg	Surface	%	Coloration	%
Products	IV	mm.sup.2	decrease	OD	decrease
Control	--	102.7 .+-. 4.46	--	1.149 .+-. 0.086	--
D 600 (two deaths/6)	1	62.6 .+-. 7.07	-39	0.664 .+-. 0.099	-42
Ginkgolide A	1	64.3 .+-. 4.2	-37	0.631 .+-. 0.054	-45
Ginkgolide B	1	58.5 .+-. 4.85	-43	0.628 .+-. 0.048	-45
Ginkgolide C	1	68.1 .+-. 6.24	-34	0.701 .+-. 0.066	-39

56 The Ginkgolides were without any effect on hyperpermeability induced by 5-hydroxy tryptamine 10 .mu.g.

57 4.2. PAF Acether-induced shock in the rabbit

58 Method: Intravenous injection of PAF Acether in unanaesthetized New-Zealand rabbits weighing 2-2.5 kg induces the increase in vascular permeability leading to plasma leakage and shock: at the highest dose, some animals present bronchospasm, convulsions and die (for details see M. SANCHEZ-CRESPO, F. ALONSO, P. INARREA, V. ALVAREZ and J. EGIDO-Vascular actions of Synthetic PAF Acether in the rat: Evidence for a platelet independant mechanism Immunopharmacology 1982, 4, 173-185).

59 Injections of PAF Acether (3-4 nmol/kg) was performed into the ear vein (in 1

ml/kg of NaCl 0.9% containing 10 mg/ml of Blue Trypan dye).

60 The extravasation was quantified at time 30 mn by measuring dye concentration in the plasma (optical density at 590 nm) after blood take from the central ear artery.

61 Treatment: Tested compounds (Gingkolides A, B and C) or vehicle were perfused in the ear vein in 45 ml NaCl 0.9% for 1 hour before PAF Acether injection. Vehicle consisted of 1.5 ml of propylene glycol completed to 45 ml by isotonic NaCl buffer; Ginkgolides were administered at the dose of 2 mg/kg IV; the figures reported in the following table are the average values of the 10 animals of each batch.

PROTECTIVE EFFECT OF GINKGOLIDES
ON PAF ACETHER-INDUCED SHOCK IN THE RABBIT

Treatment	PAF Acether (nmol/kg)	Extravasation (O.D.)	Behaviour
Control (vehicle)	--	0.896	--
Control (PAF)	2	0.991	1 animal died (convulsions)
	4	1.051	7 animals died (convulsions, bronchospasm)
Ginkgolide A	2	0.903	No death
	4	0.881	No behavioural
Ginkgolide B	2	0.827	Modifications
	4	0.880	
Ginkgolide C	2	0.910	
	4	0.875	

62 4.3. PAF Acether-induced bronchoconstriction in the anesthetized guinea-pig

63 Method: (For details, see B. B. VARGAFTIG, J. LEFORT, F. WAL, M. CHIGNARD and M. C. MEDEIROS-Non-steroidal anti-inflammatory drugs of combined with anti-histamine and anti-serotonin agents interfere with the bronchial and platelet effects of PAF Acether-Enr. J. Pharm. (1982) 82 121-130). Male Hartley guinea-pig (400-500 g) were anesthetized with urethane 2 g/kg Ip., tracheotomised and ventilated by mean of a respiratory pump: 70-80 strokes mn, stroke volume 5 ml, a pneumothorax was done to abolish spontaneous respiration. The right jugular vein was catherized and used for injections.

64 The initial resistance was kept constant at 10 cm H.sub.2 O pressure according to the method of Konzett and Rossler and the overflowed airway was measured with a bronchospasm transducer UGO BASILE connected to a UGO BASILE recorder "GEMINI". Bronchial sensitivity of the animal was checked with acetylcholine (10-40 ng kg.sup.-1 IV) and after constant response was obtained, there were injected, IV, first the propylene glycol and, five minutes later, 60 ng/kg of PAF Acether, which gave the control bronchoconstriction expressed as % of maximal bronchoconstriction given by clamping off the trachea. 40 minutes later, the tested compounds were administered by IV injection and, 5 minutes later, 60 ng/kg of PAF Acether by the same route. This experimentation was conducted on three batches of each 10 animals: first batch for Ginkgolide A, second one for Ginkgolide B and last one for Ginkgolide C, all at the 1 mg/kg dose. The figures

of the following table are the average values for the 10 animals of each batch.

Batch	% of bronchoconstriction	% of inhibition
Solvent	83.6	84.1
Ginkgolide A	13.3	
Solvent	80.2	92.3
Ginkgolide B	6.2	
Solvent	79.4	78.6
Ginkgolide C	17.0	

65 Preliminary clinical trials in shock

66 An open study has been performed on 13 thermal-injured patients (total burnt surface area >50%) treated by Ginkgolide B.

67 It has been recently shown that leukotriene and PAF Acether seem to be involved in fluid escape, oedema and anergy which characterize the thermal injury (BRAQUET Monique et al., Lancet, in Press; Comptes-rendus de l'Academie des Sciences, in Press).

68 The Ginkgolide B was administered in a perfusion (5 mg per hour for six hours and then 2 mg per hour for the following 48 hours). It has been noticed that this treatment leads to a faster recovery (decreased oedema, decreased plasma leakage and improvement of clinical outcome).

69 TOXICITY

70 The LD.sub.50 was not reached at the dose of 600 mg/kg per os on mice and rats: these compounds have a very low toxicity especially if this value is compared to the doses to be used.

71 PRESENTATION-POSOLOGY

72 For human use, these compounds may be presented in tablets and gelatine capsules for oral use, in phials for IV administration or in spray for inhalation. The following figures indicate daily doses.

73 (a) Oral route:

74 In human therapy, when using the most active of these compounds, Ginkgolide B, oral doses are from 10 to 150 mg; if Ginkgolides A or C are used, corresponding doses are from 20 to 250 mg and if Ginkgolide M is used, from 50 to 300 mg.

75 Preferred form for oral use comprises gelatine capsules containing 20 mg of Ginkgolide B or corresponding doses of the other Ginkgolides.

76 (b) IV route:

77 In human therapy, when using the most active of these compounds, Ginkgolide B, IV route doses are comprised between 1 and 20 mg; if Ginkgolides A or C are used, corresponding doses are from 2 to 50 mg, and if Ginkgolide M is used, from 5 to 70 mg.

78 The preferred dosage when Ginkgolide B is used are phials of 2 ml of isotonic solution containing 2 mg of said Ginkgolide (corresponding doses of Ginkgolides

A, C or M may be substituted to the 2 mg of Ginkgolide B).

79 (c) Spray route:

80 In human therapy, when using the most active of these compounds, Ginkgolide B, the spray-canister for 200 sprays may contain 200 mg of Ginkgolide associated with the appropriate propulsing gas; if Ginkgolides A or C are used, the spray-canister should contain 250 mg of Ginkgolide and if Ginkgolide M is used, it should contain 400 mg.

CLAIMS:

What is claimed is:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a ginkgolide or a pharmaceutically acceptable derivative thereof, the ginkgolide being present in an amount effective to treat a PAF Acether-induced malady.

2. The pharmaceutical composition of claim 1 wherein the ginkgolide is selected from the group consisting of Ginkgolide A, Ginkgolide B, Ginkgolide C and Ginkgolide M.

3. The pharmaceutical composition of claim 1 wherein the ginkgolide is Ginkgolide B, the composition is adapted for oral administration, and the Ginkgolide B is present in the amount of from 10-150 mg.

4. The pharmaceutical composition of claim 1 wherein the ginkgolide is Ginkgolide B, the composition is adapted for intravenous administration, and the Ginkgolide B is present in the amount of from 1-20 mg.

5. The pharmaceutical composition of claim 1 wherein the ginkgolide is Ginkgolide B, the composition is adapted for spray inhalation, and the Ginkgolide B is present in an amount of about 1 mg. per spray dose.

6. A method of treating or preventing a PAF Acether-induced malady in a human or animal in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of a ginkgolide or a pharmaceutically acceptable derivative thereof to treat the PAF Acether-induced malady.

7. A method of treating or preventing a PAF Acether-induced malady in a human in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of a ginkgolide selected from the group consisting of Ginkgolide A, Ginkgolide B, Ginkgolide C, and Ginkgolide M, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced malady.

8. A method of treating or preventing a PAF Acether-induced malady in a human in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of a ginkgolide selected from the group consisting of Ginkgolide A, Ginkgolide B, and Ginkgolide C, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced malady.

9. A method of treating or preventing a PAF Acether-induced malady in a human in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of Ginkgolide B, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced malady.

10. A method of treating or preventing a PAF Acether-induced malady in an animal in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of a ginkgolide selected from the group consisting of Ginkgolide A, Ginkgolide B, Ginkgolide C, and Ginkgolide M, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced

malady.

11. A method of treating or preventing a PAF Acether-induced malady in an animal in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of a ginkgolide selected from the group consisting of Ginkgolide A, Ginkgolide B, and Ginkgolide C, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced malady.

12. A method of treating or preventing a PAF Acether-induced malady in an animal in need of such treatment comprising the administration in a pharmaceutically acceptable carrier of 1 to 50 mg/kg of Ginkgolide B, or a pharmaceutically acceptable derivative thereof, to treat the PAF Acether-induced malady.